Bacterial biomass, production and heterotrophic activity of the coastal seawater at Gravelines (France)

Bacteria
Heterotrophic activity
Glucose
Epifluorescence
Production
Seawater
Bactéries
Activité hétérotrophe
Glucose
Epifluorescence
Production
Eau de mer

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Received 9/11/78, in revised form 1/2/79, accepted 3/4/79.

ABSTRACT

Bacteriological investigations carried out since 1976 in the coastal environment of the future nuclear power station at Gravelines, Northern France, have included the measurement of bacterial heterotrophic activity and biomass. The total number of bacteria, determined by epifluorescence microscopy, varied between 1.5×10^5 and 5.2×10^7/ml. As a rule, 90% of the cells showed a green fluorescence with acridine and were supposed alive, but only 2.3%, on average, were found "viable" on seawater agar. The uptake of glucose generally followed the Michaëlis-Menten kinetics, but on some occasions, results did not fit the typical saturation curve, especially during phytoflagellate (*Phaeocystis*) blooms. Nevertheless, this measure remains suitable for investigation of the effects of thermal pollution, because heterotrophic potential varied widely (by three orders of magnitude) and was significantly linked to temperature. In an attempt to determine what part of the total bacterial production resulted from the heterotrophic activity, we measured total production from the increase of biomass, by epifluorescence, in diffusion chambers without predators. From a series of 24 experiments with untreated, heated or chlorinated seawaters, it appears that the total bacterial production averages up to 16 times the maximum net uptake of glucose-C.

Oceanol. Acta, 1979, **2**, 3, 317-324.

RÉSUMÉ

Biomasse, production, et activité hétérotrophe bactériennes de l'eau de mer côtière à Gravelines (France)

Au cours d'études bactériologiques entreprises depuis 1976 en zone côtière dans l'environnement du futur site nucléaire de Gravelines (Nord, France), la biomasse et l'activité hétérotrophe bactériennes ont été mesurées.

Le nombre total de bactéries, déterminé par examen microscopique en épifluorescence, a varié entre $1{,}5.10^5$ et $5{,}2.10^7$/ml. La proportion de cellules à fluorescence verte, supposées vivantes, a toujours été de 90% environ, avec 2,3% seulement en moyenne, de germes « viables » sur milieu gélosé à l'eau de mer. L'absorption du glucose a généralement suivi la cinétique de Michaëlis-Menten, mais en certaines occasions les résultats n'ont pas correspondu à une courbe typique de saturation, notamment au cours de « blooms » de phytoflagellés (*Phaeocystis*). Malgré cela, cette mesure garde son intérêt pour l'étude des effets d'une pollution thermique, car le potentiel hétérotrophe varie largement (de 1 à 1 000), et en relation significative avec la température. Pour essayer de déterminer la part de l'activité hétérotrophe dans la production bactérienne totale, on a mesuré celle-ci par l'accroissement de biomasse en épifluorescence, en chambre à diffusion hors prédation. D'une série de 24 expériences avec des eaux brutes ou chlorées ou échauffées, il ressort que la production bactérienne totale serait environ 16 fois plus importante que l'absorption nette maximale de carbone à partir du glucose.

Oceanol. Acta, 1979, **2**, 3, 317-324.

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INTRODUCTION

Among planktonic organisms, bacteria are generally considered as "decomposers", and their abundance is an index of potential activity. They also play an important trophic role. Their biomass therefore merits an adequate description, but it has long been known that culturally "viable" bacteria in seawater account for only a small part of the total (ZoBell, 1946). Such methods as microscopic direct counts (Jannasch, Jones, 1959), adenosine triphosphate dosage (Holm-Hansen, Booth, 1966), and autoradiography (Hoppe, 1976) have been applied in attempts to estimate the total biomass of bacteria.

On the other hand, bacterial activities, which take the form of detritus removal accompanied by nutrient recycling and production of available biomass, may be altered under sublethal stress without parallel changes in the number of bacteria (Takahashi, Ichimura, 1971; Wirsen, Jannasch, 1974). Recent studies have taken this factor into account, with measurement of the uptake-velocity of several organic solutes and, occasionally, mineralization rate (Hobbie, Crawford, 1969; Williams, 1970; Wirsen, Jannasch, 1974; Gocke, 1975).

Nevertheless, the extent to which heterotrophic activities contribute to total bacterial production is not well documented, and the validity itself of the ^{14}C-assays of heterotrophic potential has been recently questioned by Sieburth *et al.* (1977) who proposed the determination of total production from biomass increase in diffusion culture.

We report in this paper the results of estimations made of bacterial biomass, heterotrophic activity and production, which were obtained during microbiological investigations in progress in the coastal environment of Gravelines (Northern France), where a nuclear power plant (3 600 MWe) will use seawater for cooling purposes.

A field study, performed before the power plant becomes operational, included measurements of biomass by epifluorescence enumeration and colony counts, and of heterotrophic activity determined by glucose uptake and mineralization experiments. Glucose-C maximum uptake and production estimated from biomass increase in diffusion culture were compared in laboratory experiments.

METHODS

Field study, biomass

The field study included weekly sampling of 3 l of seawater at the pier along which thermal effluents will be discharged. Analyses began within 15 minutes of sample collection.

For "viable-count", a portion was serially diluted with sterilized seawater and plated on modified ZoBell agar (Morita, ZoBell, 1955) in duplicate. Plates were incubated for 3 weeks at 18-20°C.

The basic method for epifluorescence enumeration was that described by Pugsley and Evison (1974). 5 to 10 ml seawater were filtered through a black membrane (Millipore HABP 0.45 µm), stained 5 minutes with 10 ml of a 1/30 000 acridine orange solution in 0.1 M phosphate buffer, pH 7.4. After 5 minutes at 37°C, the membrane was examined under oil at 1 000 × magnification. The Leitz Orthoplan microscope used was fitted with a high pressure 200 W mercury vapour lamp. Red and green fluorescent bacteria were counted separately in 20 fields, and the percentage (hereafter GFR) of cells exhibiting a green fluorescence calculated.

Heterotrophic activity

100 ml portions of 60 µm-sieved seawater were distributed into five 250 ml serum bottles. Glucose was added up to 1, 5, 10, 15 and 20 µg/l, using 0.1 µCi U-^{14}C-glucose. A parallel series of bottles was $HgCl_2$-treated (0.1 ppm, 15 minutes) and served as controls. Initial radioactivity was measured by liquid scintillation [2 ml water + 4 ml Lumagel (1) in Milli-6 (1) vials]. The bottles were closed with rubber stoppers and incubated in the dark at the *in situ* temperature, for 3 to 24 hours, so that 1 to 10% of the initial radioactivity was taken up.

Absorption was stopped by injecting 0.5 ml 1N H_2SO_4. Respired $^{14}CO_2$ was collected, as described by Thomson and Hamilton (1974), by bubbling for 30 minutes in a scintillation vial containing 0.5 ml phenethylamine, 9 ml Lipoluma (1) and 0.5 ml Lumasolve (1).

Acidified water was filtered under 100 mm vacuum and the incorporated radioactivity was measured after dissolving the membrane in 10 ml of scintillation mixture (Kennedy, 1969).

With correction coefficients applied to the incorporated fraction ($K_a = 1.05$ for quenching and recovery correction) and to the mineralized fraction ($K_r = 0.90$ for overall quenching, chemiluminescence and recovery correction, as determined with $NaH^{14}CO_3$), 97 to 98% of the initial radioactivity was recovered.

Turn-over times were calculated for each glucose concentration (mean dispersion coefficient for five sets of ten replicates : 0.14), and maximum uptake-velocity (V_M) and natural turn-over time (B) were automatically computed, according to the method of Wright and Hobbie (1965) in which V_M is the reciprocal of the slope, and B the intercept with the *y*-axis, of the best linear fit of turn-over times versus added glucose concentrations. When the linear regression was not significant over the whole range of concentrations, the initial, linear portion alone was considered.

Laboratory studies

Bacterial production and glucose uptake were compared in a laboratory continuous flow model, where chlorination and thermal shock could be applied to simulate entrainment in a power plant and subsequent dilution in the sea.

(1) LUMAC, Paris, France.

Seawater, pumped near the laboratory (mean travel time θ_R 3 minutes), reached a 50 l tank where natural production of untreated water could be measured. At the outlet of the tank, chlorine (1 ppm) was injected by volumetric pump and, after reaction for 3 minutes in a 12 l tank, the water came into contact with two 3000 W electrical plungers regulated to develop within 6 seconds a thermal shock, $\Delta_0 T$, adjustable between +0 and +20°C. Treated water then reached a cascade of eleven 50 l tanks where its temperature and eventual chlorine residue gradually decreased ($\Delta T/\Delta_0 T$ after 1 hour : 0.5; after 7 hours : 0.1) as the result of dilution at each step with adjusted flows of untreated seawater.

Production

Production in the warm tanks simulating the receiving environment was measured as for untreated water, i. e., a sample was filtered (Nuclepore 3 µm) to remove predators, and returned to the original tank, in a diffusion chamber. Biomass increase over 24 hours was determined by epifluorescence microscopy.

The diffusion chambers (20 ml) were built after McFeters and Stuart (1972) out of a piece of polymethacrylate tubing (45 mm internal diameter) fitted with two filter membranes (Millipore 0.45 µm) sealed with silicone paste. The assembly was immersed near the propeller stirring the water in each tank.

For the computation of daily production P_j, an average carbon content of 2×10^{-11} mg per bacterium (ZoBell, 1963) was used, with the following formula :

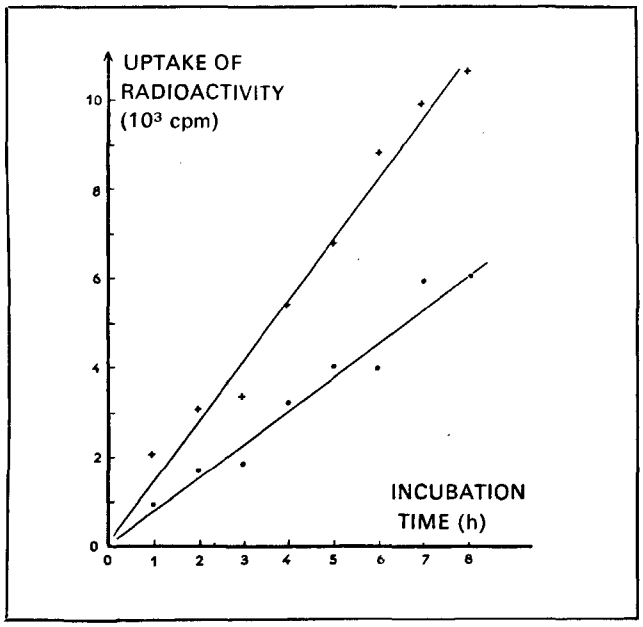
$$P_j = N.Ln\left(\frac{n_t}{n_0}\right),$$

where N is the epifluorescence number in the raw sample, n_0 and n_t being initial and final epifluorescence numbers in the diffusion culture.

Figure 1

Incorporated (+) and mineralized (●) radioactivity from ^{14}C-glucose (1 µg/l) in a seawater sample as a function of incubation length (12 April 1978; 21°C).

Radioactivité incorporée (+) et minéralisée (●) à partir de glucose ^{14}C (1 µg/l) dans un échantillon d'eau de mer, en fonction du temps d'incubation (12 avril 1978; 21°C).



RESULTS

Preliminary results of heterotrophic activity analyses

As shown in Figure 1, a good linearity between uptake and incubation time was obtained, at least until 3.5% of the initial radioactivity was taken up. Epifluorescence count showed no change in either total number or percentage of green-fluorescent cells during incubation. There must therefore be either no proliferation or equilibrium with natural predators, without drastic influence of the latter, as was suggested by Sieburth *et al.* (1977).

Preliminary results of epifluorescence enumeration

Preliminary experiments dealt with the reliability of epifluorescence enumeration and the significance of the percentage of green-fluorescent cells (GFR), which is thought to be related to the proportion of living cells.

In raw seawater, GFR is always around 90%, and in boiled seawater, GFR is about 0%, as was reported by Hobbie *et al.* (1977).

Table 1 shows that injection of 10 ppm chlorine resulted in a drop of GFR to small figures, comparable with survival on ZoBell agar. This was immediate, whereas Pugsley and Evison (1974) reported a delayed change.

On the other hand, in samples treated with 1 ppm

Table 1

Total numbers and green fluorescence ratios in chlorinated seawater. Chlorine is injected at t_0 and neutralized at $t_1 = t_0 + 20$ minutes. Samples are stored at 4°C until examined.

Nombres totaux et proportion de germes à fluorescence verte en eau de mer chlorée. Le chlore est injecté au t_0 et neutralisé au temps $t_1 = t_0 + 20$ minutes. Les échantillons sont conservés à 4°C jusqu'à examen.

Chlorine injected (ppm)	Time of examination	Total number/ml	Green fluorescence ratio (%)	Viable count on ZoBell agar/ml
0	t_1	4.2×10^4	84	1.5×10^4
1	t_1 + 30 min.	4.7×10^4	81	2.2×10^2 (1.46% survival)
	t_1 + 2 hrs.	4.7×10^4	83	
	t_1 + 4 hrs.	5.1×10^4	79	–
	t_1 + 5 hrs. 30 min.	5.0×10^4	81	–
	t_1 + 6 hrs.	4.7×10^4	83	–
	t_1 + 16 hrs.	4.6×10^4	76	–
	t_1 + 20 hrs.	4.7×10^4	73	–
10	t_1 + 15 min.	4.6×10^4	1.5	3.0×10^1 (0.2% survival)
	t_1 + 18 hrs.	4.5×10^4	2.6	
	t_1 + 24 hrs.	4.5×10^4	4.8	–

Table 2

Total numbers and green fluorescence ratios in seawater samples, after 10 minutes contact with chlorine, neutralization and immediate or delayed enumeration.

Nombres totaux et proportion de germes à fluorescence verte dans des échantillons d'eau de mer, après 10 minutes de contact avec du chlore, neutralisation et dénombrement immédiat ou différé.

Chlorine injected (ppm)	Just after dechlorination		24 hours after dechlorination	
	Total number/ml	GFR	Total number/ml	GFR
0	4.9×10^5	91	–	–
1.0	5.2×10^5	87	5.7×10^5	86
1.2	5.4×10^5	83	5.6×10^5	83
1.5	5.5×10^5	88	5.6×10^5	84
2.5	5.8×10^5	84	5.5×10^5	80
5.0	4.3×10^5	13	–	–
7.5	4.3×10^5	3.9	–	–
10	4.1×10^5	4.5	–	–
Mean	4.94×10^5	–	–	–
Dispersion coefficient	0.108	–	–	–

chlorine, "viable" count on ZoBell agar dropped but GFR remained high, even if these samples were stored at 4°C up to 20 hours before examination.

Table 2 shows that the GFR falls abruptly at chlorine levels between 2.5 and 5 ppm (in other experiments, this occurred between 5 and 8 ppm). So there would appear to be a threshold concentration, below which the GFR remains unaffected, even after 24 hours storage at 4°C.

Results of field experiments

Total count, colony count and heterotrophic potential were measured weekly from September 1976 to June 1977 and from March to July 1978 (Fig. 2).

Total count by epifluorescence ranged between 1.5×10^5 and 5.2×10^7/ml. An important ($\times 100$) peak was noticed in autumn for the first period. Correlation with temperature is apparent in spring for both periods, and is significant for the overall data set (Table 3). Apart from two exceptions, the green fluorescence ratio always remained close to the mean (90%).

Colony count on ZoBell agar represented on average 2.3% of the total count, with a poor correlation (Table 3). For the first period, a parallel count had been made on the same medium with seawater replaced by distilled water. There was significant correlation between the two colony-counts, though a minimum of 88% of the colony-forming units required seawater, on average.

Glucose maximum uptake-velocity ranged from 0.001 5 to 1.04 µg glucose/l hour and appears to be closely correlated to temperature. The mineralized fraction fluctuated between 6.6 and 62%.

In 45 out of the 72 determinations, the agreement between the observed kinetics and the modified Lineweaver-Burke equation (Wright, Hobbie, 1965) was statistically valid (linear regression coefficient $r \geqq 0.81$). Among the 27 other series, four demonstrated a high regression coefficient but the relation was not linear and results obtained by automatic computing were not acceptable. Most often the coefficient was unacceptable because of short turn-over times at the highest glucose concentrations, probably as a result of passive absorption by algae. Indeed, this occurred especially in March and April, during an algal bloom (25.700 *Phaeocystis* cells per cubic centimetre on 19 April 1978) (Bougard, 1978).

Experimental study : comparison between heterotrophic activity and production

Table 4 and Figure 3 show the results of 24 comparisons between maximum glucose net uptake and production measured in diffusion culture.

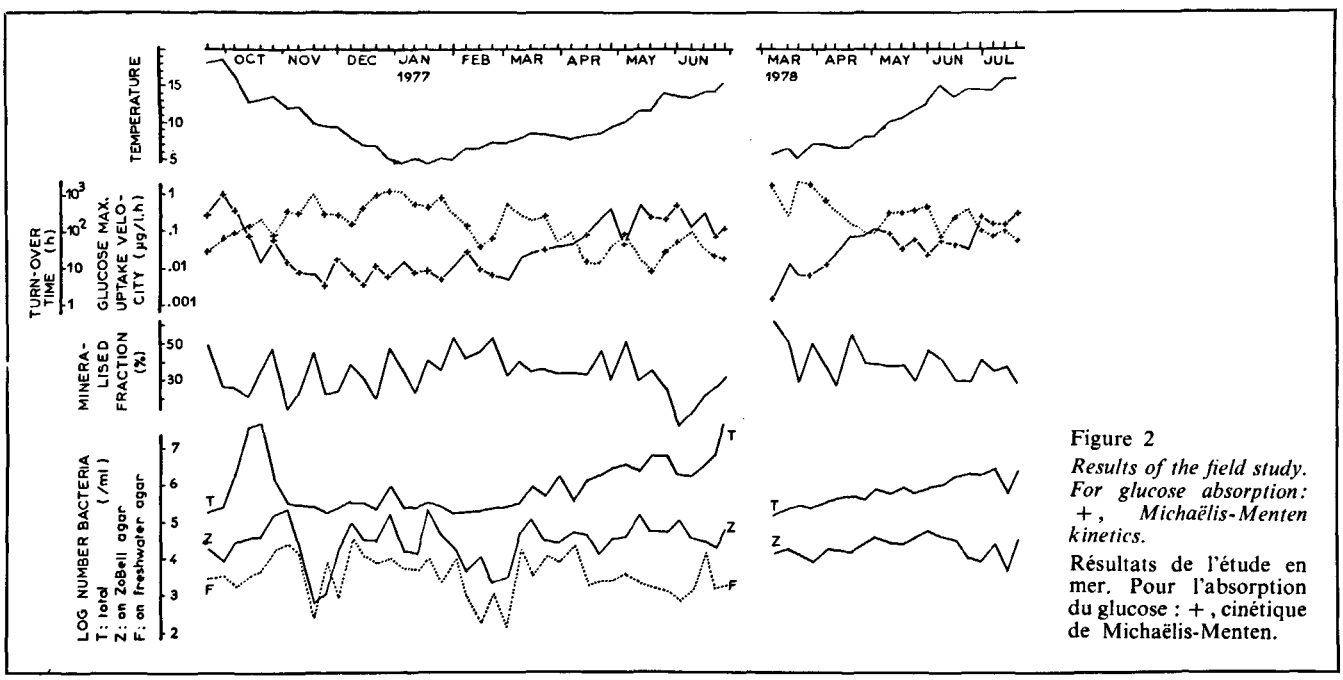


Figure 2

Results of the field study. For glucose absorption: +, Michaëlis-Menten kinetics.

Résultats de l'étude en mer. Pour l'absorption du glucose : +, cinétique de Michaëlis-Menten.

Y		X: T (°C)	log (N)/ml	log (Z)/ml	log (F)/ml	log (B) (hr.)
log N/ml	*r*	0.575		0.345	−0.011	
	a	0.096	–	0.382	−0.012	–
	b	5.013		4.329	6.076	
log Z/ml	*r*	0.151			0.644	
	a	0.023	–	–	0.642	–
	b	4.115			2.157	
log F/ml	*r*	−0.012				
	a	−0.002	–	–	–	–
	b	3.434				
log V_M (µg/l.hr.)	*r*	0.702	0.544	0.285		−0.749
	b	0.120	0.555	0.322	–	−0.756
	b	−2.635	−4.747	−2.817		0.268
log B (hr.)	*r*	−0.584	−0.627			
	a	−0.099	−0.634	–	–	–
	b	3.233	6.029			
R (%)	*r*	−0.386				
	a	−0.011	–	–	–	–
	b	0.461				
log N_r/ml	*r*			0.383		
	a	–	–	0.430	–	–
	b			3.099		

Table 3

Linear regressions between the parameters. r, coefficient; a, slope; b, constant; T, *temperature;* N, *total count;* Z, *colony count on ZoBell seawater agar;* F, *colony count on freshwater agar;* V_M, *glucose maximum uptake velocity;* B, *turn-over time;* R, *respired fraction;* N_r, *red-fluorescing fraction of the total count;* $n = 72$ *except with* F *where* $n = 52$.

Régressions linéaires entre paramètres, *r*, coefficient; *a*, pente; *b*, ordonnée à l'origine; T, température; N, nombre total; Z, nombre de colonies sur gélose de ZoBell à l'eau de mer; F, nombre de colonies sur milieu à l'eau douce; V_M, vitesse maximale d'absorption du glucose; B, temps de turn-over; R, fraction minéralisée; N_r, fraction à fluorescence rouge du nombre total : $n = 72$ sauf pour F où $n = 52$.

Figure 3

Linear regression and 5% confidence limits between production in diffusion culture and maximum glucose −C net uptake.

Régression linéaire et limites de confiance à 5% entre production en chambre à diffusion et absorption maximale, nette, de carbone à partir du glucose.

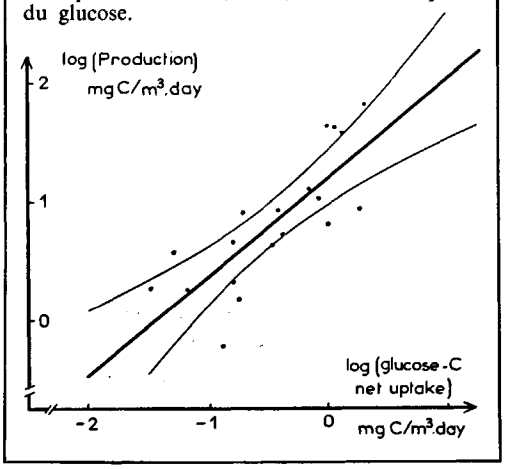


Table 4

Comparison between production rates measured in diffusion culture and with ^{14}C*-assays of heterotrophic activity.*

Comparaison entre taux de production mesurés en chambre à diffusion et par les déterminations au ^{14}C d'activité hétérotrophe.

N°	T (°C)	Production (mg C/m³.day) with ^{14}C-assays of heterotrophic activity (maximum)	Production (mg C/m³.day) in diffusion culture	Observations
1 A	8.0	0.18	1.521	Untreated
1 B	8.0	0.068	1.839	After chlorination
1 C	8.0	0.16	2.136	(residual Cl < 0.05 ppm)
2 A	6.9	0.14	0.604	Untreated
2 B	23.4	0.19	8.374	Heated
2 C	12.3	0.16	4.632	Heated
2 D	9.8	0.21	0	Heated
3 A	8.5	1.8	8.904	Untreated
3 B	14.2	0.69	13.144	After thermal shock and chlorination
3 C	12.5	0.82	10.918	(residual Cl < 0.05 ppm)
4 A	10.5	0.39	8.692	Untreated
4 B	10.5	0.033	1.903	After chlorination
4 C	10.5	0.35	4.441	(residual Cl < 0.05 ppm)
5 A	15.0	1.0	43.566	Untreated
5 B	15.0	0	0	Residual Cl : 0,3 ppm
5 C	15.0	1.3	38.266	Residual Cl < 0,05 ppm
6 A	15.0	0.41	5.459	Untreated
6 B	15.0	0.016	0	After chlorination
6 C	15.0	0.052	3.773	
6 D	15.0	0.001 3	0	
6 E	15.0	0.016	0	
6 F	15.0	1.0	6.625	Untreated
7 A	15.0	1.13	42.453	Untreated
7 B	27.9	2.0	66.250	Heated

With a chlorine residual of 0.3 ppm (Table 4, series 5 B), both glucose uptake and production are undetectable. At smaller residuals (< 0.05 ppm) production is sometimes undetectable by epifluorescence enumeration, though the more sensitive ^{14}C-assays still gives a significant value.

For the nineteen pairs of significant data, the following highly significant correlation was obtained :

log (production)

$= 0.838$ log (glucose-C net uptake) $+ 1.215$ ($r = 0.79$).

The slope is not significantly different from 1, which allows the following approximation :

production $\simeq 16 \times$ glucose-C net uptake.

DISCUSSION

Heterotrophic activity

Isotopic determination of the bacterial heterotrophic activity in seawater depends on an adequate labelling of organic matter. Extracts from natural phytoplankton cultures grown under $^{14}CO_2$ have been used (Herbland, 1975). This complex substrate probably resembles as closely as possible the natural organic matter, but this technique is far from routine. A variety of simpler substrates have been studied. The utilization of amino-acids seems particularly attractive, since they can be used in mixtures that reflect natural molar composition (Dawson, Gocke, 1978; Williams, 1976) and their natural concentration can be chemically estimated (though the results for "free" amino-acids might not reflect the "biologically available" fraction, Dawson and Gocke, 1978). However, Baross *et al.* (1975) have shown that amino-acids can be pooled by the cells before they are metabolized. Thus, what is measured in short-term experiments with amino-acids could be an overestimated (accumulation) velocity, rather than the utilization velocity.

Glucose cannot be pooled (Baross *et al.*, 1975) and can be taken up by as many bacteria as amino-acids (Hoppe, 1977), and so appears a better choice. Nevertheless, the determination of the maximum uptake-velocity is based upon the assumption (Wright and Hobbie, 1965) that there is a linear relationship between turn-over time and added glucose concentration. This is always the case in some places (Kiel Fjord, Gocke, 1975) but not everywhere (Vaccaro, Jannasch, 1967). On the site under investigation, non-linear responses were observed in about one case out of three, especially during *Phaeocystis* blooms.

Prior filtration with 3 µm Nuclepore membranes, intended to eliminate the algal interference, did not lead to linear responses. Moreover, it resulted in a 30-50% loss in bacterial number. It would also remove the predators which might contribute to the steady state of bacterial numbers observed during incubation with labelled glucose.

Despite this interference of algae, glucose uptake appears to be significantly correlated to water temperature. This determination remains useful for the study of sublethal stress such as thermal addition.

Biomass

The comparison of "heterotrophic potential" with production raises the problem of biomass determination. Cultural techniques only reveal a small "viable" fraction. A number of other techniques have been used. Among those that agree with one another (direct counts, electron microscopy, lipopolysaccharide concentration), epifluorescence appears to be quite suitable as a routine procedure. Subjective influence is not as great, as has been pointed out, and reproducibility is at least as good as for well-established cultural techniques. Accuracy has not been studied here, and would depend largely on technical details. Our results up to the present were obtained with Millipore membranes, and they should be discussed according to the findings of Hobbie *et al.* (1977) who observed in seawater twice as many bacteria with 0.2 µm Nuclepore as with Millipore membranes.

Acridine epifluorescence enumeration provides additional information, with respect to type of fluorescence. Pugsley and Evison (1974) considered the green fluorescent cells to be viable and the red ones as dead. Hobbie *et al.* (1977) state that the green fluorescent bacteria are dormant, the red fluorescence corresponding to a dominance of single-stranded nucleic acids and either RNA rich, active cells or dead cells with degraded DNA.

In our field results, the red fraction was on average fourfold greater than colony-count, and no relationship could be established with respect to viability or dormancy. Nevertheless, in a body of water where the percentage of green-fluorescent cells (GFR) is always very high, a decrease of GFR associated with chlorination or thermal shock may be reasonably considered as the expression of a lethal effect. This holds true with strong (10 ppm, 10 minutes) chlorination, but our results suggest that viability on nutrient agar can be lost, at smaller doses, without any change of GFR.

Production

Bacterial production can be estimated using the following general formula (Zaïka, *in* Hussenot, Laurent, 1973) :

$$P = N_t - N_0 + M_t$$

where M_t is the number of bacteria taken up by predators. Curds and Cockburn (1968) proposed for the predation velocity :

$$\frac{dM}{dt} = k_1 . Pr . \frac{N}{N + \alpha},$$

where k_1 is a constant characteristic of the predator-prey system, Pr is the biomass of predators, and α the value of N (number of bacteria) for which predation velocity is half the maximum.

Where N is very high, α can be neglected and if Pr is a constant, then :

$$M = k_1 . Pr . t.$$

The constant (k_1.Pr) can be derived from diffusion culture experiments without predation, by the formula of Romanova and Zonov as was done for an eutrophic freshwater pond by Hussenot and Laurent (1973).

In seawater, N is not so high, and values for α as high as 10^9/ml have been reported (Hamilton, Preslan, 1970). Thus it seems more reasonable to suppose $N \ll \alpha$. Then :

$$\frac{dM}{dt} = \frac{k_1 . Pr}{\alpha} . N.$$

If N may be considered a constant, in equilibrium with an equally constant biomass of predators, hypotheses which seem reasonable in seawater at least over a period of 24 hours, then :

$$P = M = \frac{k_1 . Pr}{\alpha} . N . t.$$

Assuming an exponential growth in diffusion culture without predators with a growth rate k, then :

$$\frac{k_1 . Pr}{\alpha} = k = \frac{Ln(n_t) - Ln(n_0)}{t},$$

where n is the number of bacteria in diffusion culture without predators.

Hence the formula wa have used in this study :

$$P = N . Ln\left(\frac{n_t}{n_0}\right).$$

In addition to the problems associated with the validity of the above assumptions and with biomass determinations, questions arise concerning measurement of growth rate. Do 3 µm filters remove all predators? Are not some nutrients removed? The concentration of dissolved nutrients probably reflects the natural one, even better in diffusion chambers (McFeters and Stuart, 1972; Sieburth *et al.*, 1977) than in dialysis bags. But if dissolved state is necessary for uptake by bacteria, it is known that organic particles can be a site of attachment (Kaneko, Colwell, 1975) with active dissolution, uptake and production. Such particulate nutrients, and the associated bacterial species, are removed by filtration.

Thus the conversion factor of × 16 observed for maximum glucose-C net uptake and production appears to be an underestimation for two reasons : use of suboptimal membranes in epifluorescence enumeration; and probable influence of filtration on growth rate in diffusion cultures.

CONCLUSION

Bacterial biomass at Gravelines was determined by epifluorescence microscopy. With this simple and rapid technique, the total count averaged up to forty times the culturally "viable" count, and seasonal variations of biomass could be appreciated, which did not appear with cultural techniques.

Epifluorescence also showed a high (90%) and constant proportion of cells exhibiting a green fluorescence, thus probably alive but inactive, which is in agreement with the small proportion of "viable" bacteria.

In the assays of heterotrophic activity with ^{14}C, the assimilation of glucose-C did not always follow a typical saturation curve, especially because of algal interferences. Nevertheless, the maximum uptake velocity appeared to be closely related to temperature, rather then to viable or even total count. Thus, this method would remain useful to appreciate the effects of thermal addition. On the other hand, the mineralized fraction varied widely without relation to known factors.

Limited comparison of glucose-C maximum uptake with production measured from biomass increase in diffusion culture showed a significant correlation between the two paremeters, but with a conversion factor of × 16. Thus, the importance of bacterioplankton production in the fertility of oceans might be much greater than previously shown by the ^{14}C-assays of heterotrophic activity. This "paraprimary" production, arising mainly from the organic excretion from phytoplankton, could account for a significant part of the photosynthetic production, not measured in the ^{14}C-bicarbonate of primary production.

Aknowledgements

This investigation was carried out as part of a co-operative programme supported by Electricité de France and the Centre National pour l'Exploitation des Océans. Valuable technical assistance from Mr. J. P. Jospin and Miss E. Lepercq is gratefully acknowledged.

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